

Figure S1. B cells are required for HDM-specific Th2 cytokine production by lung CD4⁺ T cells. (Related to Figure 1).

(A-D) B6 and μ MT mice were i.n treated with 25 μ g of HDM for 4 consecutive days starting on day 1. On day 15, mice were i.n challenged with 25 μ g of HDM daily for 4 days. The frequency and number of IL-4 (A and B) and IL-13 (C and D) producing CD4⁺ T cells in the lungs were determined on day 20 by intracellular staining after restimulation for 4 h with anti-CD3 and BFA. As a control, naïve non-treated B6 mice were also analyzed. (E-F) HDM-sensitized B6.CD45.1⁺ recipient mice were i.n challenged on day 15 with 25 μ g of HDM daily for 4 consecutive days and analyzed on day 20. The frequency (E) and number (F) of host IL-4⁺ CD4⁺ T cells were determined by intracellular staining after restimulation with anti-CD3+ BFA for 4 h. *P < 0.001 vs. HDM-treated B6. (unpaired Student's t test). Data are representative of three independent experiments (mean and S.D. of 4-5 mice per group).

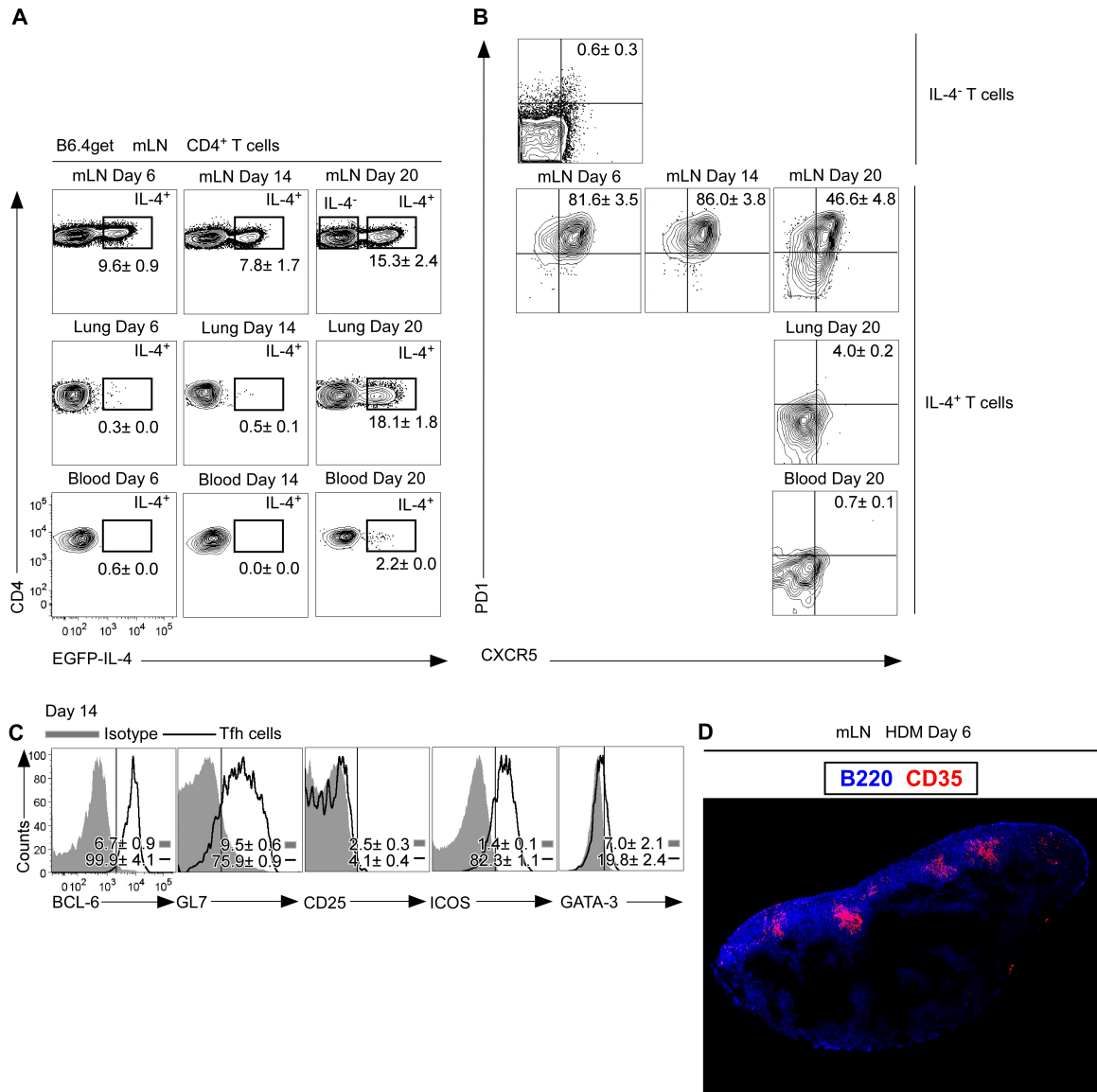


Figure S2. Kinetic analysis of IL-4-expressing (EGFP⁺) CD4⁺ T cells after administration of HDM (Related to Figure 4).

(A-C) B6.4get mice were i.n HDM sensitized on day 1 and analyzed on day 6 and day 14 or HDM sensitized on day 1, challenged on day 15 and analyzed on day 20. The frequency of IL-4-expressing (EGFP⁺) on the CD4⁺ T cells from the mLNs, lungs and peripheral blood are shown (A). Expression of PD-1 and CXCR5 on EGFP⁺ and CD44^{hi}EGFP⁺ CD4⁺ T cells are shown (B). Expression of BCL-6, GL7, CD25, ICOS and GATA-3 on CD44^{hi}EGFP⁺ CD4⁺ T cells in the mLN of HDM-treated mice are shown at day 14 (C). (D) B6 mice were i.n HDM sensitized on day 1 and analyzed on day 6. Cryosections from the mLN were stained with anti-B220 (blue) and CD35 (red) and analyzed by fluorescent microscopy. Data are representative of two independent experiments (mean and S.D. of 4-5 mice per group).

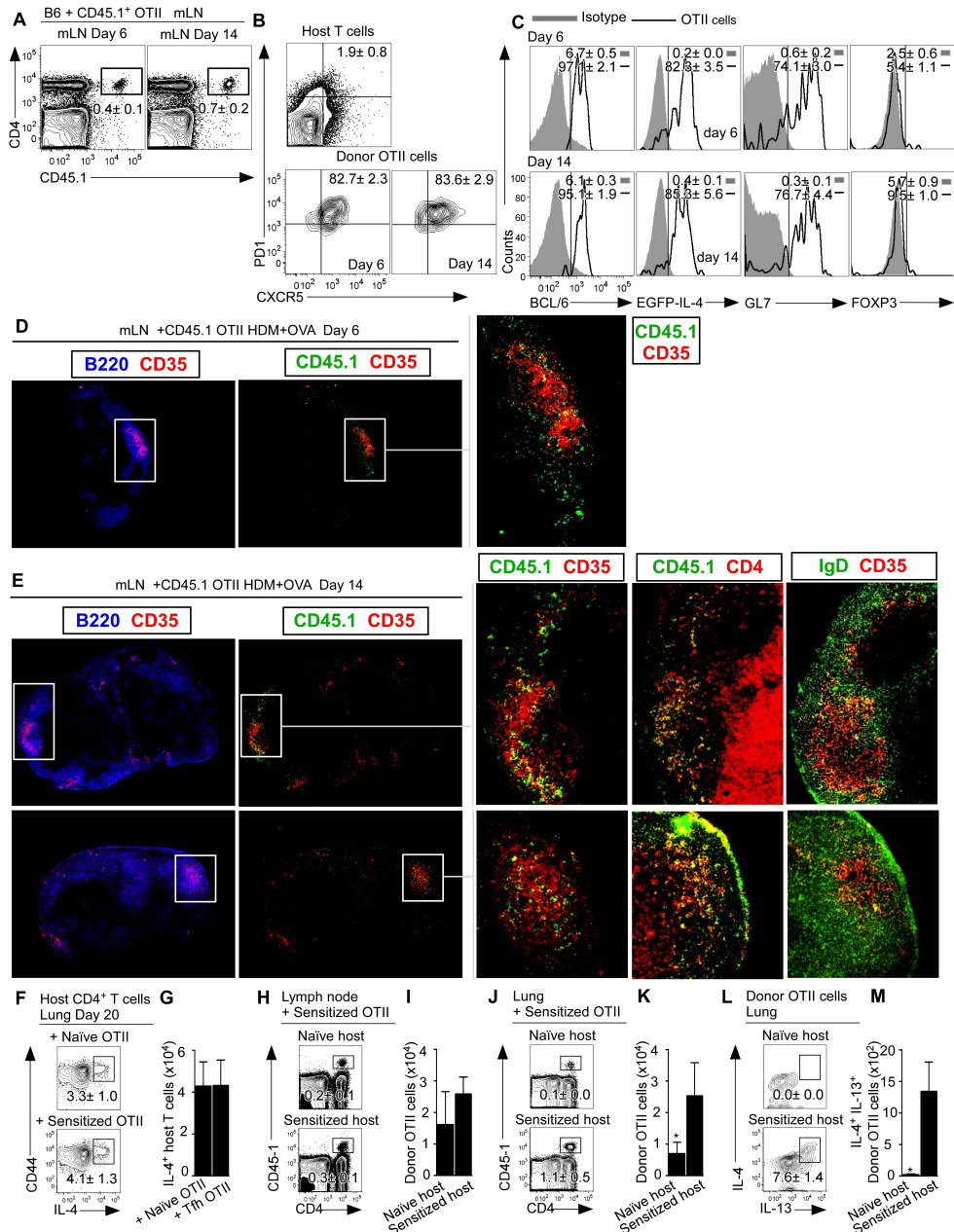


Figure S3. Kinetic analysis of the phenotype and intranodal localization of donor OTII cells after sensitization (Related to Figure 5).

(A-C) 25×10^3 naïve OTII. 4get. CD45.1⁺. CD4⁺ T cells were transferred into naïve B6 mice. One day later, recipient mice were i.n treated with HDM+OVA for 4 consecutive days and analyzed 2 days after the last HDM inoculation. Frequency of donor OTII T cells from the mLN on day 6 and 14 (A). Expression of CXCR5 and PD1 (B) and BCL-6, EGFP-IL-4, GL7 and FOXP3 (C) in donor OTII T cells from the mLN on day 6 and 14.

(D-E) 25×10^3 naïve OTII. CD45.1⁺. CD4⁺ T cells were transferred into naïve B6 mice. One day later, recipient mice were i.n HDM+OVA sensitized and analyzed on day 6 (D) and day 14 (E). Cryosections from the mLN were stained with anti-B220 (blue), CD35 (red) and CD45.1 (green), with anti-B220 (blue), CD4 (red) and CD45.1 (green) or with anti-B220 (blue), CD35 (red) and IgD (green) and analyzed by fluorescent microscopy. (F-G) HDM-sensitized B6 recipient mice were i.n challenged on day 15 and analyzed on day 20. The frequency (F) and number (G) of host (CD45.2⁺) IL-4 producing CD4⁺ T cells in the lungs were determined by intracellular staining after restimulation for 4 h with anti-CD3 and BFA. (H-M) 25×10^3 OTII CD45.1⁺ CD4⁺ T cells obtained from hosts 6 days after HDM+OVA sensitization were transferred into naïve B6 mice or day 6 HDM+OVA-sensitized B6 mice. 8 days later, recipient mice were i.n treated with HDM+OVA for 4 consecutive days and analyzed 2 days after the last HDM inoculation. The frequency (H,J) and number (I,K) of donor CD45.1⁺ OTII T cells in the mLN (H-I) and lungs (J-K) are shown. The frequency (L) and number (M) of IL-4/IL-13-producing cells among the donor CD45.1⁺ OTII T cells were determined in the lungs by intracellular staining after restimulation for 4 h with anti-CD3 and BFA. *P < 0.01 (unpaired Student's t test). Data are representative of two or more independent experiments (mean and S.D. of 4-5 mice per group).

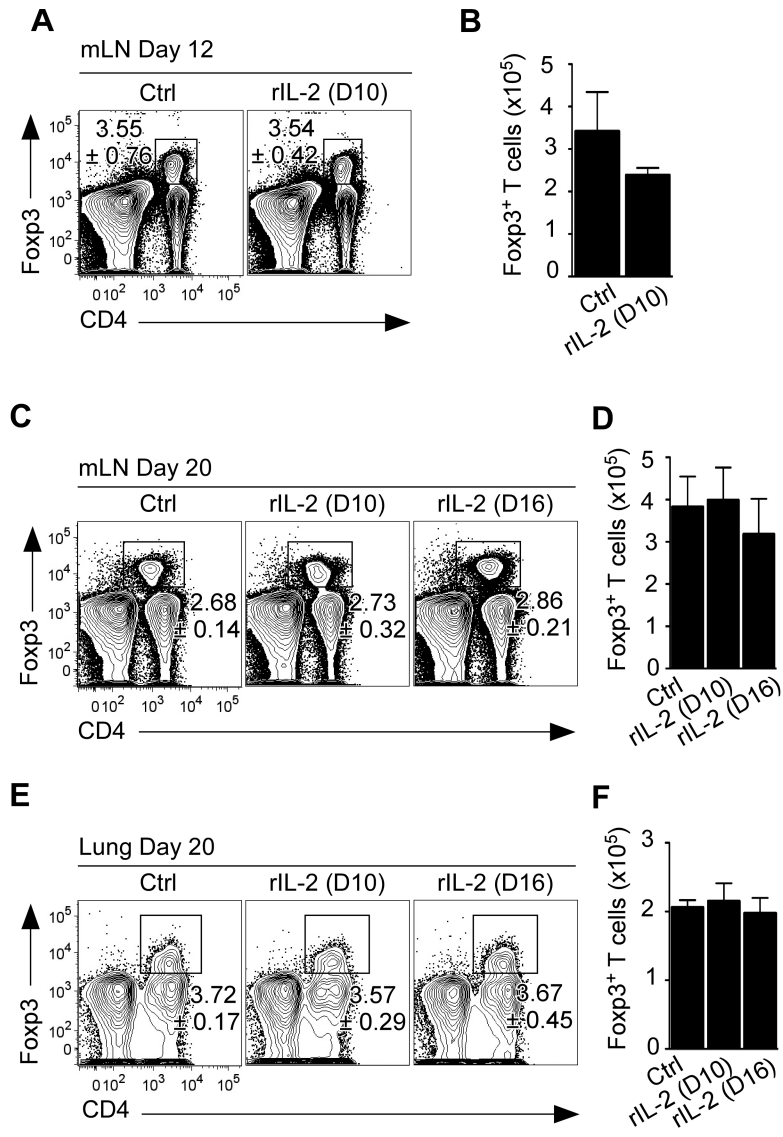


Figure S4. rIL-2 treatment in HDM-treated mice does not increase the number of Foxp3⁺ Treg cells (related to Figure 7).

(A-B) Frequency (A) and number (B) of host Foxp3⁺ Treg cells from day 12 mLN of HDM+OVA-treated B6 mice injected with rIL-2 or PBS according to the schematic in **Figure 7E**. (C-F) Frequency and number of host Foxp3⁺ Treg cells from day 20 mLN (C-D) and lungs (E-F) of HDM-treated B6 mice injected with IL-2 or PBS according to the schematic in **Figure 7E**.